

Vascular Resistance in the Isolated Gills of *Octopus macropus* and *Nautilus pompilius*¹

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ABSTRACT: The gills of cephalopods represent a potential site of regulation of vascular peripheral resistance. Measurements of pressure–flow relationships in the isolated gills of *Octopus macropus* and *Nautilus pompilius* gave no evidence of autoregulation. Perfusion with putative neurotransmitters showed 5-hydroxytryptamine, and possibly dopamine, to reduce vascular resistance. Physiological concentrations of acetylcholine and noradrenaline did not alter resistance to flow.

THE CEPHALOPOD VASCULAR SYSTEM parallels the vertebrate vascular system in many of its morphological features. Johansen and Martin (1962) suggested that the cephalopod system could be separated into seven functional elements that have counterparts in the vertebrate system. Included in these elements are the resistance vessels, composed of the termination of the arterial system, and the exchange vessels, made up of the capillaries and sinuses. Collectively, these comprise the microvasculature, and there appear to be many differences between the structures of the cephalopod and vertebrate microvasculature (Barber and Graziadei 1965, Kawaguti 1970). In the vertebrates, changes in vascular peripheral (hydraulic) resistance at the level of the microvasculature (mostly the arterioles) are of paramount importance in the control of regional as well as overall blood flow. A central question, then, is: To what extent does the function of the cephalopod microvasculature differ from that of the vertebrates? This question can be answered in part by examining pressure–flow relationships and the concomitant peripheral resistance that

exists in isolated vascular beds perfused by a Newtonian fluid (Green, Rapela, and Conrad 1963).

During the R/V *Alpha Helix* expedition to the Philippines, we examined the patterns of pressure–flow relationships in isolated preparations of gills from *Octopus macropus* and *Nautilus pompilius* to gain insight into what physical factors are important in peripheral resistance control. We also investigated the effects of some putative cephalopod neurotransmitters on the pressure–flow relationships in these isolated gill preparations.

MATERIALS AND METHODS

Freshly caught *Octopus macropus* and *Nautilus pompilius* were placed in large seawater aquariums and maintained for at least 24 hr before use. The afferent ctenidial vessel of a gill was cannulated in situ after lightly anesthetizing the specimen with 3% ethanol (*Octopus*) or 2% urethane (*Nautilus*) in seawater. The polyethylene tubing (PE 100, 0.86 mm I.D.) used as the catheter was connected via rubber tubing passed through a peristaltic pump to a 250-ml glass container.

Cannulated gills were removed from the specimen and placed in seawater in a circular plastic container 20 cm in diameter. A central drain with a vertical glass tube kept the water level constant at 3 cm depth. This water was continuously aerated.

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Perfusion flow rates were monitored by two means simultaneously. The perfusion reservoir was suspended from a calibrated Grass FT 10 force transducer and the weight of the container was continuously plotted on a Brush 2400 four-channel recorder. A Biotronix 2-mm flowthrough-type electromagnetic flow probe powered by a Biotronix 610 flowmeter, was also placed in the perfusion line near the gill. The output of the Biotronix 610 flowmeter was routed to a second channel of the Brush recorder. At the low flow rates used in this study, the change in weight of the reservoir proved to be the most sensitive measure of flow. Millipore filtered seawater was selected as a perfusion fluid for two reasons: (1) adequate volumes of octopus and nautilus blood were not available, and (2) seawater behaves as a Newtonian fluid. Preliminary viscosity studies indicate that octopus blood, with considerable amounts of dissolved protein, does not behave as a Newtonian fluid. It is likely that this is true of nautilus blood as well.

Input pressures to the gill were followed with a Statham P23 Db pressure transducer connected by a T-tube to the perfusion canula near the inlet to the gill. This transducer was calibrated daily against a distilled water manometer. Pressure measurements were recorded on a third channel of the Brush recorder.

Flow rates were controlled by a variable-speed peristaltic pump (Cole-Parmer Masterflex). At a given flow rate, an increase in input pressure indicated an increase in vascular resistance in the gill and, conversely, a decrease in input pressure signalled a drop in vascular resistance.

The effects of certain possible neurohumors on vascular resistance were examined by perfusing these substances through the gills at a constant flow rate and noting any changes in input pressures. Solutions of these substances were prepared in millipore filtered seawater. Pressure-flow experiments involved five *Octopus macropus* (wet wt 355 ± 73.4 g) and six *Nautilus pompilius* (wet wt 634 ± 168.8 g). Drug experiments were performed on five *Octopus* (wet wt 249 ± 85.3 g) and eight *Nautilus* (wet wt 574 ± 131.9 g). All experiments were conducted at $25 \pm 1^\circ\text{C}$. All measures of variability are expressed as standard deviations.

RESULTS

At a constant perfusion flow rate the pressure in each gill tested varied with time in a characteristic manner. At the inception of perfusion the hydraulic resistance was relatively low and, consequently, a low input pressure

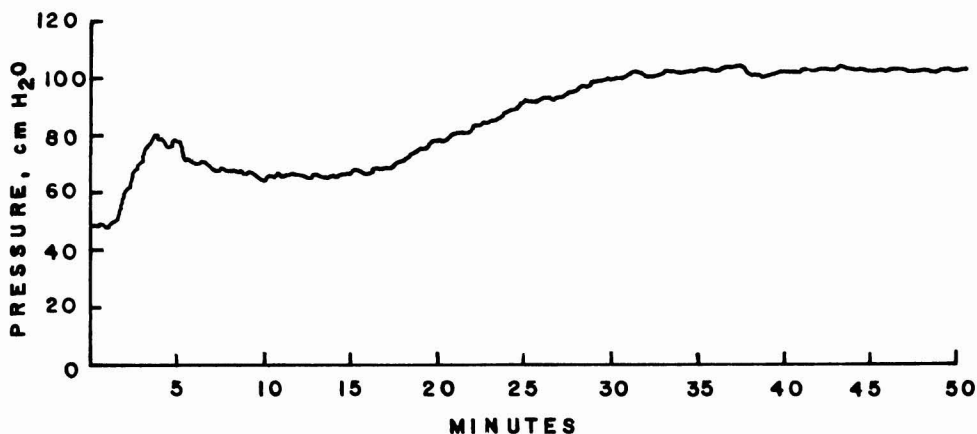


FIGURE 1. Vascular resistance as a function of time in an excised gill from *Octopus macropus*. Perfusion rate was constant at $8.6 \text{ ml} \cdot \text{min}^{-1}$.

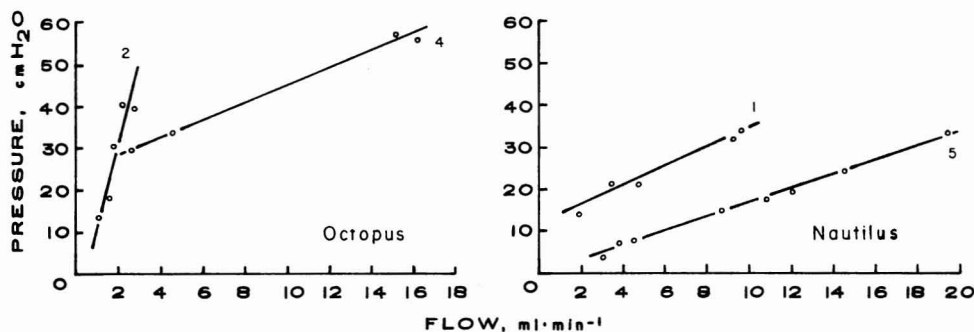


FIGURE 2. Pressure-flow relationship in the excised gill of two *Octopus macropus* (#2 and 4) and two *Nautilus pompilius* (#1 and 5).

appeared. The pressure then rose, peaked at a moderately high value, and then dropped again but not as low as the original value. This reduction in vascular resistance was short-lived and pressure again gradually increased, often reaching high values before stabilizing at about 30 min (Figure 1). The preparation then showed a very slight but ever-increasing resistance over the next 2 hr. After this increase, there was a rapid deterioration of the preparation characterized by rapidly increasing hydraulic resistance. Absolute pressures varied considerably among specimens and input pressures were, of course, higher at higher flow rates. The highest pressures (indicating highest resistance) occurred in octopus gills. The same pattern of events occurred in the nautilus gills, but the pressures that developed were not as great.

After reaching the stable point, flow rates were varied and the corresponding input pressures measured. Over the range of pressures and flows used in these experiments the pressure-flow relationship was almost linear rather than the more common curvilinear one (Figure 2). This relationship varied greatly among the octopus gills but averaged $0.32 \pm 0.14 \text{ ml} \cdot \text{min}^{-1} \cdot \text{cm H}_2\text{O}$ (range: 0.03–0.76). Variation was not as great in the nautilus gills and the corresponding average was $0.45 \pm 0.09 \text{ ml} \cdot \text{min}^{-1} \cdot \text{cm H}_2\text{O}$ (range: 0.13–0.71). Using Fick estimates of cardiac output published by Johansen, Redmond, and Bourne (1978) and Wells (1979) and assuming that blood is equally distributed to each gill, an approximation can be made of

the amount of blood that would be expected to pass through each gill at normal perfusion pressures. These values are $7.82 \text{ ml} \cdot \text{min}^{-1}$ for a pressure difference across the gill of 5 cm H_2O in a 355-g octopus and $0.79 \text{ ml} \cdot \text{min}^{-1}$ for a pressure difference of 2 cm H_2O in a 634-g nautilus. The magnitude of flow for the in vitro situation is clearly much below the expected in vivo flow rates for the octopus gill but in the same range as the expected value for the nautilus gill.

Furthermore, the pressure-flow profiles in Figure 2 demonstrate that vascular beds in both the octopus and nautilus gills are quite distensible. For example, for nautilus #1 gill there was better than a twofold decrease in peripheral resistance (where peripheral resistance, $\text{PR} = \text{Pressure}/\text{Flow}$) over an 18-cm H_2O pressure range. The octopus gill behaved in like manner; in octopus gill #4 peripheral resistance was almost halved over a linear increase in pressure of 20 cm H_2O .

In addition to developing greater vascular resistance during perfusion, the octopus gill differed from the nautilus gill in that the former developed a severe edema in the region adjacent to the branchial gland (i.e., the attached edge of the gill). The most likely reason for the edema was a high filtration rate of water from the branches of the afferent vessel that form part of the blood supply to the branchial gland.

Since the octopus gill normally operates with a small back-pressure between it and the heart (Johansen and Martin 1962), the effect of a 2-cm H_2O back-pressure applied to the

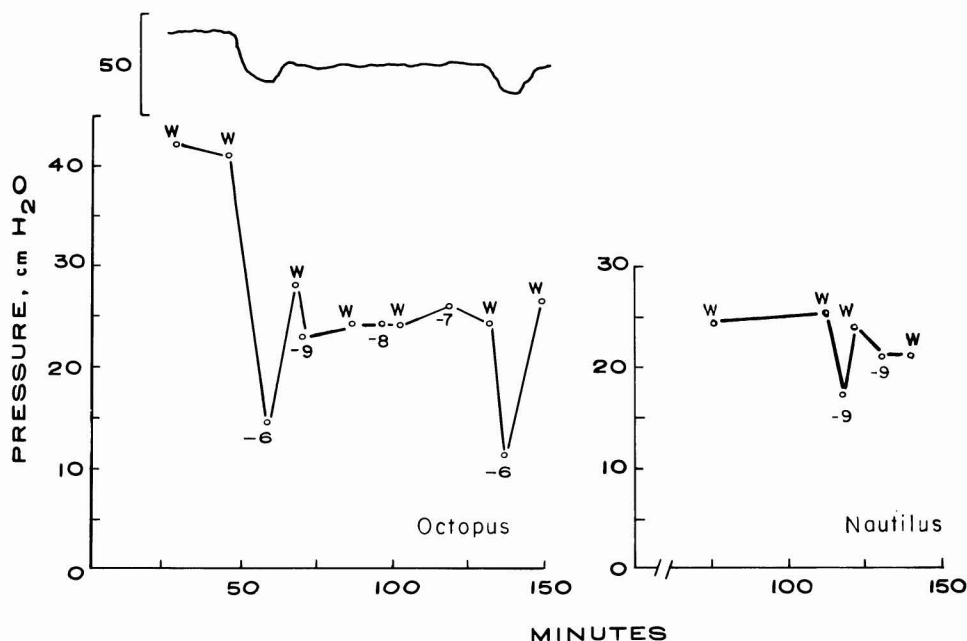


FIGURE 3. The effect of 5-hydroxytryptamine on the vascular resistance of excised gills of *Octopus macropus* and *Nautilus pompilius*. The upper left insert is a reduced drawing of the recorded response of the *Octopus* gill. Negative numbers indicate concentrations of 5-HT (e.g., -6 = 10^{-6} M); W represents perfusion with filtered seawater only. Perfusion rates: *Octopus*, $3.8 \text{ ml} \cdot \text{min}^{-1}$; *Nautilus*, $5.5 \text{ ml} \cdot \text{min}^{-1}$.

effluent ctenidial vessel was tested. This had no apparent effect on the high vascular resistance measured in the octopus gill.

Gills were perfused with some putative cephalopod neurohumors dissolved in seawater in order to gain possible insights into control of gill vascular resistance. Concentrations of 10^{-8} to 10^{-5} M acetylcholine did not alter the resistance of the octopus gill nor did 10^{-8} M concentration affect resistance of the nautilus gill. At 10^{-3} M, acetylcholine caused the octopus gill to contract, increasing resistance to flow. This is believed to be a pharmacological response to abnormally high concentrations of acetylcholine rather than a physiological response. Noradrenaline at 10^{-6} M similarly had no effect upon resistance of the nautilus gill; it was not tested in the octopus.

5-Hydroxytryptamine (5-HT), on the other hand, had a marked effect on vascular resistance of the gills of both species. In *Nautilus*, 10^{-9} M 5-HT clearly reduced gill vascular resistance. Higher concentrations (10^{-6} M) were required for a similar effect in *Octopus*

(Figure 3). At 10^{-5} M, the effect on the nautilus gill was strikingly reversed, with resistance sharply increasing (Figure 4). As often happens in drug tests, it frequently was not possible to reverse completely the effects of an application of a drug by washing. Consequently, the results are complicated by the effects of one or more previous drug applications influencing the response to a succeeding application.

Preliminary results of two experiments with dopamine indicate that this substance, at 10^{-8} – 10^{-6} M, may also reduce vascular resistance in the nautilus gill, although the effect does not seem as great as that of 5-HT.

DISCUSSION

One recurrent problem throughout these experiments, due in part to our limited equipment aboard ship, was our inability to maintain a preparation for a long enough period to permit repeated pressure–flow profiles. Addi-

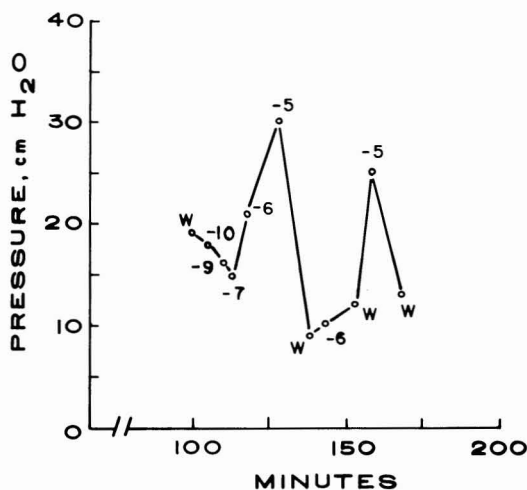


FIGURE 4. The effect of 10^{-5} M 5-hydroxytryptamine on the vascular resistance of an excised gill of *Nautilus pompilius*. Negative numbers indicate concentrations of 5-HT (e.g., $-5 = 10^{-5}$ M); W represents perfusion with filtered seawater only. Perfusion rate: $5.7 \text{ ml} \cdot \text{min}^{-1}$.

tionally, poor viability of the gills could have been caused by the use of filtered seawater as the perfusate. As explained previously, our choice of this perfusate was prompted by a desire to use a Newtonian fluid that would clear our results of complications caused by any anomalous viscosity characteristics of cephalopod blood.

The initial low peripheral resistance at the start of perfusion could have been caused by a locally triggered vasodilation in response to hypoxia or to the anesthetic used during cannulation. Although no actual measurements were made of oxygen tension, the manner of preparation of the isolated gills was such that the gills could conceivably become hypoxic for a short period prior to perfusion. The origin of the slight vasodilation prior to stabilization could not be ascertained. The increasing vascular resistance that characterized the decay of the preparation has been observed in squid systemic vascular beds (Bourne 1979) and in fish gills (e.g., Keys 1931, Wood 1974). It might simply represent rigor contractions of the vasomotor systems as the preparations lose their viability.

The difference in behavior of the nautilus and octopus gills during perfusion can be

accounted for on anatomical grounds. In nautilus there is no branchial gland running along the dorsal edge of the gill as there is in octopus. The circulation arrangement in the branchial glands of octopus is peculiar in that these glands receive blood from branches of afferent branchial vessels as well as from arteries arising from the aorta. Blood is drained from the branchial glands by veins leading to the lateral venae cavae (Isgrove 1909). The edema and higher vascular resistance of the octopus gill was probably due to either drainage or filtration of perfusate from the afferent branches into the branchial gland.

The basic relationship between pressure and flow in both nautilus and octopus gills was a linear one rather than the curvilinear relationship observed by many mammalian workers (see Green et al. 1963). A curvilinear relationship between pressure and flow has been described by Bourne (1979) for part of the systemic vasculature in squid. Mammalian workers have ascribed the curvilinear relationship to passive distensibility in "non-reactive" or "passive" tissues, i.e., vascular beds that do not show autoregulation of vasomotor tone (Green et al. 1963, Kuida 1965). The linear relationship seen here appears to indicate that the isolated octopus and nautilus gills could be included in the category of passive tissues. The other type of vascular bed, the autoregulating kind, typically shows sigmoid pressure-flow relationships, a situation seen in some fish gills (Wood 1974) but not in any of our preparations.

Our experimental arrangement did not permit us to look for a critical closing pressure in the cephalopod gills. This pressure is one below which flow would not occur because of collapse of the vascular channels (Nichols et al. 1951).

The chemical agents used (acetylcholine, dopamine, 5-HT, and noradrenaline) are suspected neurotransmitters of cephalopod central nervous systems (see Tansey 1979 for a review of cephalopod neurotransmitters). The action of these substances on isolated molluscan hearts is well known (Hill and Welsh 1966). Much less is known about the role of these putative neurotransmitters in the rest of the circulatory system. Although acetyl-

choline appears to have no physiological action on the isolated gill of nautilus and octopus, it does cause a dose-dependent vasodilation in the isolated, posterior systemic circulation of squid (Bourne 1979). Johansen and Huston (1962) found that acetylcholine acted as a peripheral vasodilator in intact *Octopus dofleini* but with less potency than noradrenaline. The reason that high doses of acetylcholine (10^{-3} M) cause contraction and increased peripheral resistance of octopus gills is not known at the present time.

Like acetylcholine, noradrenaline had no effect on the peripheral resistance in the isolated nautilus gill. This lack of gill vascular responsiveness to noradrenaline seems to be a major difference between cephalopod gills and fish gills. In isolated fish gills, the catecholamines, primarily adrenaline, cause an α -vasoconstrictor effect superimposed on a more dominant β -vasodilator effect (Payan and Girard 1977).

Of the four putative neurotransmitters, 5-HT caused the greatest response in the cephalopod gill and thus is a candidate for a role in normal vasomotor control. This assessment is based on the relatively low concentration of 10^{-9} M 5-HT that caused vasodilation in the nautilus gill. To date, the major role assigned to 5-HT in the cephalopod cardiovascular system is one of cardioacceleration and stimulation of cardiac contractility (Johansen and Huston 1962). However, the vasodilatory effect on the gill is not surprising because the same factors that would call for cardiac augmentation would necessitate increased blood flow through the gills; hence, the lower gill peripheral resistance. 5-Hydroxytryptamine has been found in the gills of other mollusks. In the gastropod, *Aplysia*, 5-HT has been localized in the gill (Perez and Estes 1974) and has been shown to stimulate cyclic adenosine-3', 5'-monophosphate (cyclicAMP) production in slices of gill (Kebabian, Kebabian, and Carpenter 1979). The physiological role of 5-HT in *Aplysia* gill is not understood (Kebabian et al. 1979). In bivalves, e.g., *Mytilus* and *Modiolus*, 5-HT has been shown to stimulate particle transport by cilia (Gosselin and O'Hara 1961). These studies indicate that 5-HT might have a physiological

role in controlling different aspects of gill function in the three major classes of mollusks, but more study is needed before a full understanding of this role is possible.

The other putative cephalopod neurotransmitter that showed vasodilatory activity in the nautilus gill was dopamine. Time limitations prevented testing in the octopus gill. The apparent difference in sensitivity of the nautilus gill to 5-HT and dopamine might simply be caused by the much higher rate of autooxidation of dopamine, thus obscuring its effective dose. It is generally known that dopamine is especially sensitive to autooxidation in alkaline media. Thus, while the isolated gills of octopus and nautilus do not show evidence of autoregulation, it appears likely that nervous control of gill vascular resistance exists in the intact animals.

The fact that both dopamine and 5-HT produced the same action in the nautilus gill has parallels in the bivalve, *Mytilus edulis*, where exogenous 5-HT and dopamine both stimulate frontal ciliary activity of the gill (Malanga 1975). Dopamine is also known to be active in the gill of the gastropod, *Aplysia*, where it causes asynchronous movements of efferent vessel trunklets, pinnate longitudinal muscles, and afferent vessels (Swann, Sinback, and Carpenter 1978). In the nautilus gill it is possible that 5-HT and dopamine might be acting at a composite dopamine/5-HT receptor. This type of receptor was first postulated by Woodruff (1971) to account for dopamine and 5-HT activity in certain cells of the *Helix aspersa* brain. However, more research is necessary before we can accept a composite dopamine/5-HT receptor as the active site causing the similarity of action of dopamine and 5-HT in the nautilus gill.

This study of pressure-flow relationships in isolated gills of nautilus and octopus lends further weight to the contention by Bourne, Redmond, and Johansen (1978) that the nautilus cardiovascular system is functionally more similar to the coleoid system than it is different. The few differences that were observed appear to have an anatomical basis. Although the linear pressure-flow relationships of the cephalopod gills are analogous to those of fish gills, the cephalopod gills show

clear molluscan affinities in the types of putative neurotransmitters that have vasomotor activity.

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